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Rearing Black Soldier Fly to Supplement Natural Populations in Waste Composting Systems

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REARING BLACK SOLDIER FLY TO SUPPLEMENT NATURAL POPULATIONS IN WASTE COMPOSTING SYSTEMS

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ABSTRACT

The black soldier fly, *Hermetia illucens* L. (Stratiomyidae; Diptera), is a common and widespread non-pest fly and an essential decomposer of organic material. Because of its ability to decompose wastes and return nutrients to the environment, it is considered to be a beneficial insect for manure management in confined animal facilities. Larvae are an excellent source of protein and other nutrients and are used for fish and animal feed. The development of efficient rearing systems for black soldier fly is important in order to provide a source of larvae to initiate soldier fly-based waste-recycling systems and to augment populations in existing systems. However information on black soldier fly rearing is relatively limited. The objectives of this study were: (1) to compare different media to identify those that result in optimal black soldier fly pupation rates and to track effects on subsequent adult emergence and oviposition, and (2) to determine the influence of moisture level in the pupation media on adult emergence. Three different pupation media were evaluated in the first experiment; compost, mulch and vermiculite. The results indicated that adult emergence rates were not significantly different among the different pupation media suggesting that all three media are suitable for pupation. A second experiment was done to evaluate the influence of moisture in mulch pupation

media. In this experiment, the average number of adults emerging from the medium and high moisture level treatments were significantly greater than in the low moisture treatment. In addition, the overall total adult male and female emergence rates were also significantly higher in the high and medium moisture treatments compared to the low moisture treatment. These results suggest that moisture levels in pupation media should be maintained between 50-85% to achieve optimum adult emergence rates. However, about 10% of emergent adults in the high moisture level treatment had malformed wings. Therefore, moisture levels between 50-55% may be ideal to achieve optimum adult emergence without the occurrence of abnormal wing development. Successful adult mating and oviposition was achieved in commercially available BugDorm^{TM} tents kept inside a greenhouse with supplemental lighting. These results demonstrate that successful black soldier fly mating and oviposition can occur in small cages with sufficient numbers of adults present and if they are kept in a greenhouse under the conditions described herein.

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Literature Review

The black soldier fly, *Hermetia illucens* L. (Stratiomyidae; Diptera), is a common and widespread fly with a geographic distribution that includes most of the western hemisphere (McCallan, 1974). The black soldier fly has also spread through human activity to Australia and New Zealand (McCallan, 1974). Unlike other fly species, neither the adult nor larvae are considered pests or vectors of disease (Sheppard et al., 1994). Rather, they are beneficial in that they are essential decomposers of organic material (Sheppard et al., 1994).

Black soldier fly normally undergo three generations per year in their natural habitat (Sheppard et al., 1994) and commonly occur in decomposing organic waste (Tomberlin et al., 2009) including animal manure (Sheppard, 1983). They are considered to be a beneficial insect in confined animal facilities because of their ability to decompose waste and return nutrients to the environment. Larvae are an excellent source of protein and other nutrients and are used for fish and animal feed (Newton et al., 1977, Sheppard et al., 1994). Previous studies have shown that black soldier fly can convert poultry manure into larval biomass as an animal feedstock containing 42% protein and 35% fat with an 8% dry matter conversion rate (Sheppard et al., 1994). This system reduced total manure

volume by 50% (Sheppard, 1983) and reduced total nitrogen mass and total nitrogen concentration by 62% and 24%, respectively (Sheppard et al., 1994).

Black soldier fly waste recycling systems have been proposed as a commercially viable method to process poultry and swine manure in confined animal feeding operations (Newton et al., 2005). As an added benefit, dense soldier fly larval populations also reduce house fly, *Musca domestica* L., production in manure by 94-100% (Sheppard 1983). Soldier flies are known to compete with house flies for larval habitat, and studies have shown that female house flies do not oviposit where soldier flies are moderately abundant, apparently due to repellant chemicals released by soldier flies (Bradley and Sheppard, 1984).

Soldier fly larvae also accelerated the inactivation of the human pathogen *Escherichia coli* O157: H7 in chicken manure, but not in cow or hog manure, apparently due to different pH conditions in chicken compared to other manures (Erickson et al., 2004).

As a feedstock, in addition to protein and fat, soldier fly larvae and pre-pupae are a good source of fatty and amino acids (Hale, 1973) and have been recommended as a replacement for soybean in animal feed (Sheppard et al., 1994).

Black soldier fly adults are dark-colored, non-biting flies 15-20 mm long and are wasp-like in appearance (Sheppard et al., 1994). Black soldier fly males and females may be distinguished by differences in the genitalia at the tip of their abdomen. The female abdomen has a scissor-shape structure at the tip that is used for mating and oviposition. The male has a fan- or plate- like structure at the tip of the abdomen (Anonymous, 2013).

Although black soldier fly develop naturally in organic material, larvae and adults become inactive under sub-optimal environmental conditions. Therefore the development of efficient rearing systems for black soldier fly is important in order to provide a source of larvae to initiate soldier fly-based waste-recycling systems and to augment populations in existing systems. However information on black soldier fly rearing is relatively limited.

Black soldier fly larvae store fat reserves during development that are utilized in the adult stage; therefore adults require only water for mating and oviposition activities (Tomberlin et al., 2002, Newton et al., 2005). Tingle et al. (1975) reported that soldier fly mating and oviposition occurred in 3 x 6.1 x 1.8-m cages and in smaller 0.76 x 1.14 x 1.37-m cages held outdoors, but no mating and oviposition occurred in smaller 0.76 x 1.14 x 1.37-m cages held in a greenhouse. These authors reported that direct sunlight

encouraged mating, but neither mating nor oviposition was observed in small cages (0.53 x 0.91 x 0.53-m and 0.38 x 0.46 x 0.38) kept outdoors (Tingle et al., 1975).

Wild black soldier fly females oviposit in dry cracks and crevices above decomposing organic matter (Copello, 1926) (Gonzalez et al., 1963). Booth and Sheppard (1984), reported that females will lay eggs in the small openings at the edges of corrugated cardboard near suitable larval media such as decomposing organic matter. Mating starts 2 days after adult emergence from pupae (Tomberlin and Sheppard, 2002) and females will lay approximately 300-600 eggs after copulation and then die within hours (Tomberlin and Sheppard, 2002; Tomberlin et al., 2002). Black soldier fly larvae hatch in 4-6 days at 27℃ (Copello, 1926; Booth and Sheppard, 1984). Tomberlin and Sheppard (2002) reported that about 80% of eggs will hatch if humidity is kept at 60% and the temperature is maintained at 26℃. Seasonal differences in egg hatch was reported from a New Zealand study where the length of time for egg hatch was 5 days in February and 7-14 days in April (May, 1961).

Black soldier fly larvae will move toward a food source directly after hatching (Booth and Sheppard, 1984), and larvae feed for 10-14 days and undergo 5 instar stages before molting into pupae (Tomberlin et al., 2002). The pupation period is approximately

10-14 days at 27°-30°C (Sheppard et al., 1994). Pre-pupae migrate from the food source apparently to seek a dry, dark environment for pupation (Newton et al., 2005). After moving to an appropriate pupation substrate, pre-pupae remain on the surface for a short while before burrowing into the substrate (Weston and Desurmont, 2008; Holmes, 2010). Migrating pre-pupae evacuate their digestive tract and no longer feed, and pupation takes a minimum of 10 days (Newton et al., 2005). Because of this migratory behavior, systems can be designed to self-collect pre-pupae in the final stage and to stockpile them prior to processing or other utilization.

No studies have been done specifically to evaluate different media for black soldier fly pupation and influence on subsequent adult emergence, mating and oviposition. Also, in previous studies reporting successful rearing of black soldier fly, the rearing took place in a greenhouse with natural light (Sheppard et al., 2002). The authors recommended that future studies be done to determine if black soldier fly rearing could be done using artificial lighting in order to reduce costs.

The objectives of this study were: (1) to compare different media to identify those that result in optimal black soldier fly pupation rates and to track effects on subsequent adult emergence and oviposition, and (2) to determine the influence of moisture level in

the pupation media on adult emergence. Information from this experiment will be useful in designing small-scale black soldier fly rearing systems in a greenhouse or other indoor facility where space is limited.

Materials and Methods

Experiment I – Comparison of Pupation Media for Black Soldier Fly Development

1. Adult emergence

This experiment was initiated on July 11, 2015 at the Clemson University Greenhouse Complex inside a 3.66 m x 10.67 m temperature-controlled greenhouse. An Air King 9940 humidifier (Air King, West Chester, PA) was used to keep the humidity between 30-60% with a temperature range of 26° - 32℃. Larvae of black soldier fly, *Hermetia illucens,* were collected from two locations. One was from a wild population at the Clemson University Cherry Crossing Research Center composting facility for processing food waste from campus. The other population was obtained from the Forensic Laboratory for Investigative Entomological Sciences directed by Dr. Jeffery Tomberlin at Texas A&M University.

The rearing media evaluated for pupation and adult emergence in this experiment were mulch, compost and vermiculite. Mulch was obtained from the Cherry Crossing facility and was composed of finely chopped wood chips from the campus landscaping services. Compost media was a mixture containing 75% finely ground woodchips (mulch) and 25% food waste sourced from the campus dining hall that had been through a thermal

decomposition process. The waste included both plant-based and protein-based foods. The vermiculite media was Sta-Green™ Vermiculite (Sun Gro Horticulture Distribution Inc, Agawam, MA). Compost and mulch samples were submitted to the Clemson Agricultural Services Laboratory for analysis. Vermiculite, an inorganic silica-based material, was not subjected to analysis. Please refer to the Results section for results of the analyses.

The pupation media was placed 10.2 cm-deep inside 38.1 x 29.2 x 29.9 cm clear plastic boxes (Sterilite™ Corporation, Townsend, MA). The pupation media boxes were then placed inside larger 0.61 x 0.61 x 0.61 m plastic boxes with three sides of screened ventilation and one opening for access in order to collect the adults after emergence. Each media treatment was evaluated with three replications, and the nine rearing chambers were placed randomly on a bench inside the greenhouse (Figure 1). The media materials in all treatments were kept at the same moisture level of 50-55%; moisture was measured every other day using a VH400™ soil moisture sensor connected with a VG-METER-200™ soil moisture meter (Vegetronix, Inc., Riverton, UT) and water added using a hand sprayer if needed.

Moisture levels were taken in five random locations in each box and averaged. If

moisture levels dropped below 50-55% water was added and then the moisture levels was measured after 10 minutes to maintain desired moisture levels between 50 to 55%. Once desired moisture levels were attained, 210 black soldier fly pre-pupae were placed into the nine media boxes on July 13, 2015. Adult emergence began on July 19. Adults were removed gently by hand, counted and identified to sex, placed temporarily inside small plastic cups with screen lids, and then placed inside rearing chambers (BugDorm™ tents; see below).

2. Adult Reproduction and Oviposition System

Each pupation box was placed inside a corresponding 'rearing tent' with dimensions of 0.75 x 0.75 x 1.15-m (BugDorm$^{TM:}$ MegaView Science Co., Ltd., Taichung, Taiwan) (Figure. 2). As was done by Sheppard et al. (2002), artificial plants were placed inside each tent to serve as resting places for adults, and water was sprayed on the plants each day to provide droplets for adults to drink.

Artificial lighting was added to supplement natural light inside the greenhouse and to encourage black soldier fly mating. McIver and O'Grady (1987) reported that black soldier fly males use sunlight to locate females, and that male ommatidia in their compound eyes can detect ultraviolet light (Gates, 1980). Briscoe and Chittka (2001)

indicated that insects normally cannot see red or infrared light, and the longest wavelength that can be seen by insects is 700 nm. Zhang et al. (2010) reported that black soldier fly mating did not occur under lighting provided by a rare earth lamp at 350 - 450 nm. Therefore in the present study, artificial light in the 290 - 320 nm range was provided as a supplement to natural sunlight to encourage mating.

A POWERSUN^{TM} Vapor UVB reptile light (Zoo Med Laboratories, Sacramento, CA) was placed on the top of each tent and connected to a timer to turn on lights from 0630 to 1130, and from 1330 to 2030 each day to supplement natural sunlight. Lights were turned off from 1130 to 1330 to avoid excess heat inside cages during the hottest part of the day. To provide a surface for adult oviposition, four 5 x 8 cm pieces of corrugated cardboard were taped together in a stack and glued to the inner side of a 42.9 cm x 29.2 cm x 14.9 cm plastic box (Sterilite Corporation, Townsend, MA) and placed 3.8 - 5 cm above a food source for larval development. The food source was made using 1 cup of corn meal (Martha White Foods Inc., Memphis, TN), 1 cup of rice cereal (Malt-O-Meal, Lakeville, MN) and 2 cups of water.

3. Larval Counting Method

To estimate fecundity in each treatment, larvae were sampled from four random

locations inside each larval development box. Sampling was done approximately 9-days after initial egg hatch when most larvae were in the 3-4 instar stage. At each location a 50 ml beaker was used to collect a 20 ml volume of the food source containing larvae. The number of larvae in each 20 ml sample was counted and averaged to calculate an average number of larvae per treatment and replicate. The total volume of food source and larvae inside each box was also determined by placing the entire content of each box inside a graduated beaker. An estimate of the total number of larvae per box was calculated as follows:

$$\text{Total no. of larvae} = \frac{Avg.no.larvae\ per\ 20ml\ sample\ \times\ total\ volume\ of\ food\ source}{20\ ml\ sample\ volume}$$

4. Statistical analysis

Data were analyzed using JMP® Pro 12.0.1 statistical software. ANOVA and Fisher's least significant difference test were used to compare treatment differences in numbers of adults and larvae, larvae per adult and adult emergence rates by gender.

Because adult emergence increased initially and then tapered off, emergence data were modeled using a typical sigmoid response curve, specifically the Gompertz three parameter equation (JMP™ Version 12.0.1 Online, The Fit Curve Report). The three parameters were: asymptote (denoted a), growth rate (denoted b), and inflection point

(denoted c). The exact form of the Gompertz equation was

Adult accumulation = a × $exp\ \{-exp[-b \times (day - c)]\}$

Experiment II – Comparison of Media Moisture Levels

1. Adult emergence

This experiment was done to evaluate the effect of different moisture levels in mulch pupation media on adult emergence. Mulch media was used in this experiment because the results of Experiment I indicated that mulch yielded the highest number of adults and was comparable to compost media in adult emergence rates. In addition, results of a preliminary, non-replicated experiment indicated that adult emergence was greater in mulch and compost media with moisture levels of 50.6% and 60.6%, respectively, compared to wood chips with a low moisture level of 23.9%. These results suggested that media moisture level may be an important factor in adult emergence. Therefore, an experiment was done to evaluate different moisture levels in mulch media using similar methods to those reported for Experiment I.

In this experiment, three different moisture levels for mulch pupation media were tested: low (20-25%), medium (50-55%) and high (80-85%). The same procedures for

measuring moisture levels reported for Experiment I were used in this experiment. Media moisture level treatments were replicated three times and black soldier fly development parameters were evaluated under the same greenhouse conditions as described in Experiment I.

This experiment began on August 8, 2015. The procedures for evaluating adult emergence were the same as described for Experiment I, except that 120 pre-pupae were placed in each pupation treatment box. Media moisture levels in each box were quantified every other day to maintain correct levels. Adult emergence began on August 13, 2015.

2. Adult Reproduction and Oviposition

The same procedures described for Experiment I were used to collect, count and sex adults and to provide conditions for oviposition and development of neonate larvae.

Results

Experiment I – Comparison of Pupation Media for Black Soldier Fly Development

1. Pupation Media Analysis

Results of the laboratory analyses for samples of mulch and compost media are given in Table 1. The results indicated that the mulch and compost media differed in some key parameters, including C/N ratio, organic matter, and soluble salts.

2. Adult Emergence

Adult emergence began on July 19, 2015 and continued through August 2. Peak emergence occurred during the first 3-5 days (Figure 3). Peak adult emergence occurred on July 21, 2015 in the compost treatment and on July 23, 2015 in the mulch and vermiculite treatments.

Data on adult emergence in the different media treatments are provided in Table 2. The mulch media treatment provided the highest average adult emergence rate (82.86%), followed by vermiculite (78.1%) and compost (68.57 %); however differences were not significant ($P>0.05$) (Table 2). The cumulative adult emergence data in all media treatments demonstrated a typical sigmoidal response where emergence peaked initially and then tapered off (Figs. 5-7). The Gomperts model asymptote estimate confidence

intervals for the compost data did not overlap with mulch and vermiculite indicating that cumulative adult peak emergence in the compost media treatment was significantly lower than in the other treatments (Table 7). In addition, the inflection point, or day with the greatest number of emergent adults, occurred earlier in the compost treatment than in the other media treatments.

3. Adult Reproduction, Oviposition and Larval Development

The BugDorm™ tents provided a suitable habitat for adult reproduction and oviposition. Egg masses were laid on the cardboard strips located above the larval food source, and egg-hatch and subsequent larval development occurred in all treatments. The compost treatment yielded significantly greater numbers of larvae (average no. per 20 ml sample and total number per box) compared to the vermiculite treatment, however larval numbers in the compost and mulch treatments were not significantly different (Table 2). Similarly, larvae/adult ratios were significantly greater in the compost treatment compared to the vermiculite treatment, but compost and mulch ratios were not significantly different (Table 2). Table 3 provides data on the numbers of black soldier fly larvae produced by adults from the different pupation media; these data were subjected to statistical analyses reported in Table 2.

Experiment II – Comparison of Media Moisture Levels

1. Adult Emergence

Adult emergence began on August 13, 2015 and continued through August 25. Peak adult emergence in all moisture level treatments occurred on 19 August, 7 days after the onset of adult emergence (Figure 4).

The average number of adults emerging in the different moisture level treatments were 62.33 in the low moisture treatment, 96.33 in the medium moisture treatment, and 101.00 in the high moisture level treatment. This corresponded to average emergence rates of 51.94% in low moisture, 80.28% in the medium moisture treatment, and 84.17% in the high moisture treatment (Table 4). Total adult and male and female emergence rates were significantly higher in the medium and high moisture level treatments than in the low moisture treatment (P=0.0007). As with the media treatment data, the cumulative adult emergence data in the moisture level treatments demonstrated a typical sigmoidal response (Figs. 8-10). The Gomperts model asymptote estimate confidence intervals for the moisture data do overlap among treatments, indicating that cumulative adult peak emergence was not significantly different among treatments (Table 8). Moreover, the inflection point, or day with the greatest number of emergent adults, was similar among

moisture level treatments.

Interestingly, emergent adults, both males and females, had a significantly higher percentage of malformed wings in the high moisture treatment than in low and medium moisture level treatments ($P<0.001$, Table 6). Observations of adults with malformed wings indicated that they were unable to fly and mate successfully (Figure 11).

2. Adult Reproduction, Oviposition and Larval Development

Successful black soldier fly reproduction and oviposition was achieved in the BugDorm™ tents. Egg masses were laid on the cardboard strips located above the larval food source, and egg-hatch and subsequent larval development occurred in all moisture level treatments. Average numbers of larvae per sample and total larvae produced did not differ significantly among moisture level treatments (Table 4).

Table 5 provides data on the numbers of black soldier fly larvae produced by adults from the different pupation media; these data were subjected to statistical analyses reported in Table 4.

Discussion & Conclusion

There are several key findings from this study that will aid in design of low-cost indoor black soldier fly rearing systems where space may be limited. The commercially available BugDorm^{TM} tents with artificial lighting provided a low cost, suitable environment for adult reproduction and oviposition as described in the Results section. Furthermore, the box containing the larval food source and the cardboard oviposition strips placed inside the tents provided an effective system for female oviposition and egg hatch onto the food source. Larvae developing in the food source boxes can easily be collected for subsequent storage as a feedstock, or to supplement black soldier fly populations in active composting systems.

Tingle et al. (1975) reported that black soldier fly mating and oviposition did not occur in small cages inside a greenhouse, suggesting that larger cages may be needed. Tomberlin and Sheppard (2002) reported successful adult mating and oviposition inside larger (1.5 x 1.5 x 3-m) cages. Sheppard et al. (2002) also reported successful mating and oviposition inside larger (2 x 2 x 4-m) cages placed inside a greenhouse. The present study demonstrated that successful mating and oviposition was achieved inside small (0.75 x 0.75 x 1.15-m) BugDorm^{TM} tents. Furthermore, mating and oviposition was

observed in the smaller (0.61 x 0.61 x 0.61-m) plastic pupation boxes. These results indicate that successful mating and oviposition can occur in small cages where sufficient numbers of adults are present under the conditions that have been described herein.

The results of Experiment I indicated that adult emergence rates were not significantly different among the different pupation media (compost, mulch, vermiculite) (Table 2). This suggests that all three pupation media are suitable for adult emergence. However, larval production based on the average number of larvae and larvae/adult ratios were significantly greater in the compost compared to the vermiculite treatment, suggesting that compost may be a superior pupation media if adult fecundity is a primary objective. Analysis of mulch and compost samples indicated that the two pupation media differed in some key parameters. For example, compost had a lower C:N ratio and percentage organic matter than mulch, but was somewhat higher in soluble salts and bulk density. Because black soldier fly pupae do not feed, one would not necessarily expect that specific characteristics of the pupation media such as those described above would influence the subsequent fecundity of adults emerging from pupae in the media. However additional physiological studies are needed in order to determine the influence of pupation media on adult fecundity.

Based on results of a preliminary experiment (data not shown) indicating that moisture level in the pupation media may be a critical factor in adult emergence, Experiment II was done to evaluate the effects of moisture level on black soldier fly development using mulch media. In this experiment, the average number of adults emerging from the medium and high moisture level treatments were significantly greater than in the low moisture treatment (Table 4). In addition, the overall total adult, male and female emergence rates were also significantly higher in the high and medium moisture treatments compared to the low moisture treatment. These results suggest that moisture levels in pupation media should be maintained between 50-85% to achieve optimum adult emergence rates. However, about 10% of emergent adults in the high moisture level treatment had malformed wings and were unable to fly, indicating that these adults were not able to successfully mate and reproduce. Although the results suggest that high moisture levels in pupation media may be a factor in abnormal wing development, additional studies are needed to confirm a cause and effect relationship.

Some pre-pupae in the high moisture level media boxes were observed in attempts to crawl out of the box, and this behavior was not observed in lower moisture level treatments. This is congruent with the behavior of black soldier fly pre-pupae to move to

a drier substrate for pupation. Therefore, moisture levels between 50-55% may be ideal to achieve optimum adult emergence without the occurrence of abnormal wing development.

Analyses of adult emergence data indicate that peak adult emergence occurred earlier in compost media than in the other media treatments (Figs. 3, 5, 6, 7). This suggests that compost may be a superior media if the goal is to achieve early adult emergence, but additional studies are needed to confirm these results.

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Table 1. Results of Compost and Mulch Media Analysis[z]

	Mulch	Compost
Total Nitrogen	0.25%	0.71%
Carbon	22.30%	13.40%
C : N	90.92	18.90
OM (organic matter)	46.97%	26.9%
EC (soluble salts)	0.66 mmhos/cm	2.22 mmhos/cm
pH	5.8	6.0
Bulk density (vol basis)	1092 lb/yard	1190 lb/yard
Moisture	50.61%	60.61%

[z]Wet basis values

Table 2. Black soldier fly adult emergence from pupae, and 1st generation larval production in each pupation media treatment.

Pupation Media	Adults[v]	Ave. no. larvae per sample[w]	Total larvae[x]	L / A[y]	Emergence percentage[z]		
					Total	Male	Female
Compost	144.00A	180.08A	8141.07A	61.29A	68.57A	37.30A	31.27A
Mulch	174.67A	139.42AB	6288.63A	36.56AB	82.86A	36.83A	46.03A
Vermiculite	164.00A	82.42B	2611.73B	15.83B	78.10A	37.94A	40.16A
	$F_{2,6}$=0.8428 P=0.4758	$F_{2,6}$=8.7953 P=0.0165	$F_{2,6}$=18.3546 P=0.0028	$F_{2,6}$=6.2530 P=0.0341	$F_{2,6}$=0.8187 P=0.4849	$F_{2,6}$=0.0132 P=0.9869	$F_{2,6}$=1.5517 P=0.2863

[v]Average no. of adults per treatment; n=3

[w]Average no. larvae per 20 ml food source sample (4 samples per treatment box; 3 boxes per treatment)

[x]Total estimated no. larvae per food source box (3 boxes per treatment)

[y]L/A = larva/adult ratio

[z]Emergence percentage = $\left(\frac{adults}{prepupae}\right)$***100%**

Means in each column not sharing the same letter are significantly different (Fisher's least significant difference test, α=0.05;

ANOVA F-statistic, df=2,6, and P-values for testing the hypothesis that means are not significantly different among treatments).

Table 3. Numbers of 1st generation black soldier fly larvae produced by adults in the different pupation media treatments.

Media	Box	Sample[w] 1	Sample[w] 2	Sample[w] 3	Sample[w] 4	Avg. no. of larvae[x]	Total volume[y](ml)	Total larvae[z]
	1	107	153	168	159	146.75	1075	7887.8
Compost	2	210	235	213	209	216.75	800	8670.0
	3	162	186	198	161	176.75	890	7865.4
	1	165	171	75	134	136.25	920	6267.5
Mulch	2	187	158	166	190	175.25	920	8061.5
	3	112	108	103	104	106.75	850	4536.9
	1	94	58	83	79	78.50	450	1766.3
Vermiculite	2	84	69	73	87	78.25	730	2856.1
	3	76	96	86	104	90.50	710	3212.8

[w]Total no. larvae in each 20 ml sample of media (4 samples per box; 12 samples per media treatment).

[x]Average no. larvae per 20 ml sample per treatment box (N = 4 samples per box).

[y]Total volume of food source and larvae in each box.

[z]Estimated no. of total larvae per box (see methods for calculation in Material and Methods section).

Table 4. Black soldier fly adult emergence from pupae, and 1st generation larval production in mulch media at different moisture levels.

Moisture level	Adults[v]	Ave. no. larvae per sample[w]	Total larvae[x]	L / A[y]	Emergence percentage[z]		
					Total	Male	Female
High	101.00A	168.92A	4716.20A	56.19A	84.17 A	41.95A	42.22A
Medium	96.33A	81.58A	2413.60A	24.72B	80.28 A	41.11A	39.17A
Low	62.33B[y]	116.08A	3544.23A	46.24AB	51.94 B	27.22B	24.72B
	$F_{2,6}$=30.9203[z]	$F_{2,6}$=2.5310	$F_{2,6}$=2.0749	$F_{2,6}$=3.4330	$F_{2,6}$=30.9134	$F_{2,6}$=15.8203	$F_{2,6}$=23.2586
	P=0.0007	P=0.1596	P=0.2066	P=0.1014	P=0.0007	P=0.0041	P=0.0015

[v]Average no. of adults per treatment; n=3

[w]Average no. larvae per 20 ml food source sample (4 samples per treatment box; 3 boxes per treatment)

[x]Total estimated no. larvae per food source box (3 boxes per treatment)

[y]L/A = larva/adult ratio

[z]Emergence percentage = $\left(\frac{adults}{prepupae}\right)$***100%**

Means in each column not sharing the same letter are significantly different (Fisher's least significant difference test, α=0.05;

ANOVA F-statistic, df=2,6, and P-values for testing the hypothesis that means are not significantly different among treatments.

Table 5. Numbers of 1st generation black soldier fly larvae produced by adults emerging from pupae kept in mulch media at different moisture levels.

Moisture level	Box	Sample[w] 1	Sample[w] 2	Sample[w] 3	Sample[w] 4	Samples average[x]	Total volume[y](ml)	Total larvae[z]
	1	90	88	163	135	119.00	1075	6396.3
High	2	60	68	65	48	60.25	970	2922.1
	3	217	206	135	118	169.00	420	3549.0
	1	26	14	35	26	25.25	1042	1315.5
Medium	2	116	124	125	131	124.00	380	2356.0
	3	98	109	84	91	95.50	560	2674.0
	1	184	235	154	158	182.75	460	4203.3
Low	2	120	89	170	131	127.50	510	3251.3
	3	191	209	233	153	196.50	545	5354.6

[w]Total no. larvae in each 20 ml sample of media (4 samples per box; 12 samples per media treatment).

[x]Average no. larvae per 20 ml sample per treatment box (N = 4 samples per box).

[y]Total volume of food source and larvae in each box.

[z]Estimated no. of total larvae per box (see methods for calculation in Material and Methods section).

Table 6. Abnormal adult wing development in adults emerging from mulch media kept at different moisture levels.

Moisture level	Adults with wing defects[y]
Low	0.00B
Medium	0.00B
High	10.00A
	$F_{2,6}$=300.0000[z]
	P<0.001

[y]Means Mean no. of adults with wing defects in each media moisture level treatment. Means not sharing the same letter are significantly different (α=0.05, P<0.05, JMP 12.0.1).

[z]F-statistic, df=2,6, and p-value for testing the hypothesis that means are equal for are three media using ANOVA.

Table 7. Gompertz 3P model fit of cumulative adult emergence data over time in the three pupation media treatments.

Treatment	Parameter	Estimate	Standard Error	Lower 95%	Upper 95%
Compost	Asymptote	137.951	5.998	126.195	149.707
	Growth Rate	0.430	0.086	0.261	0.599
	Inflection Point[z]	2.854	0.323	2.220	3.488
Mulch	Asymptote	175.370	8.988	157.754	192.985
	Growth Rate	0.335	0.055	0.226	0.443
	Inflection Point	4.731	0.315	4.115	5.348
Vermiculite	Asymptote	168.368	2.688	163.100	173.636
	Growth Rate	0.394	0.023	0.349	0.439
	Inflection Point	5.059	0.096	4.870	5.247

[z]Inflection Point = Indicates day of peak adult emergence.

Table 8. Gompertz 3P model fit of cumulative adult emergence data over time in the media moisture level treatments.

Treatment	Parameter	Estimate	Std Error	Lower 95%	Upper 95%
High	Asymptote	108.428	4.018	100.554	116.303
	Growth Rate	0.359	0.038	0.284	0.434
	Inflection Point[z]	4.663	0.189	4.293	5.033
Medium	Asymptote	69.416	3.776	62.014	76.817
	Growth Rate	0.306	0.041	0.226	0.386
	Inflection Point	4.735	0.284	4.179	5.291
Low	Asymptote	102.855	4.695	93.652	112.058
	Growth Rate	0.378	0.053	0.273	0.482
	Inflection Point	4.432	0.237	3.967	4.897

[z]Inflection Point = Indicates day of peak adult emergence

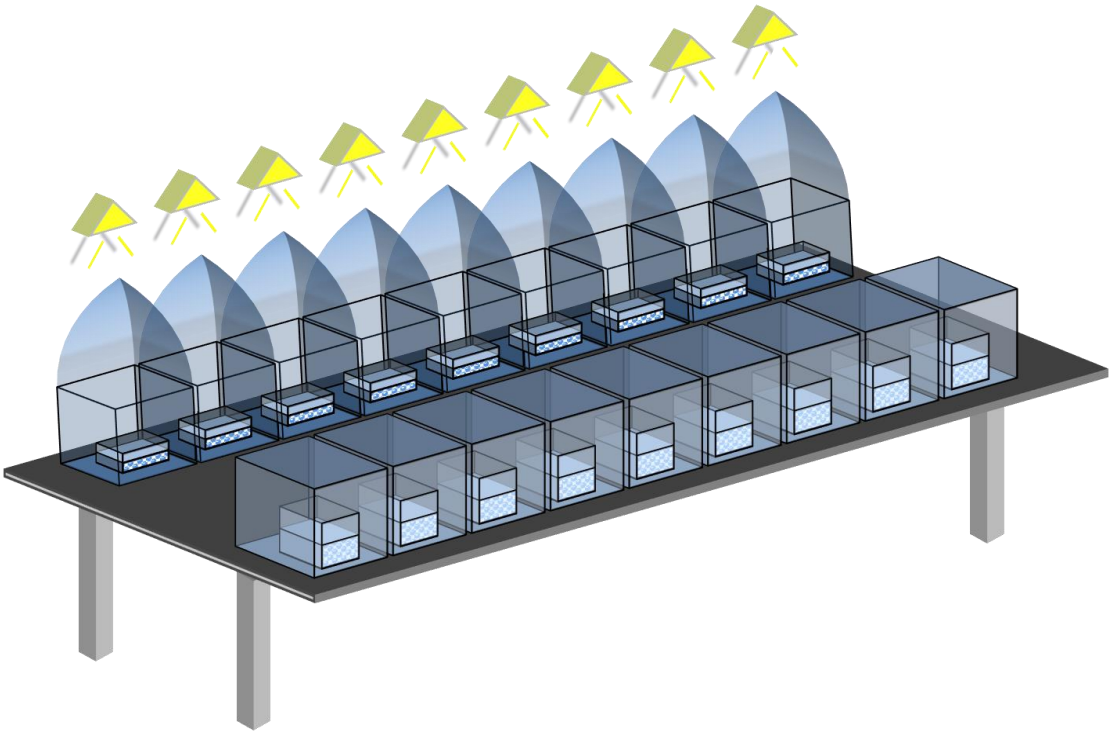

Figure 1. Experimental arrangement

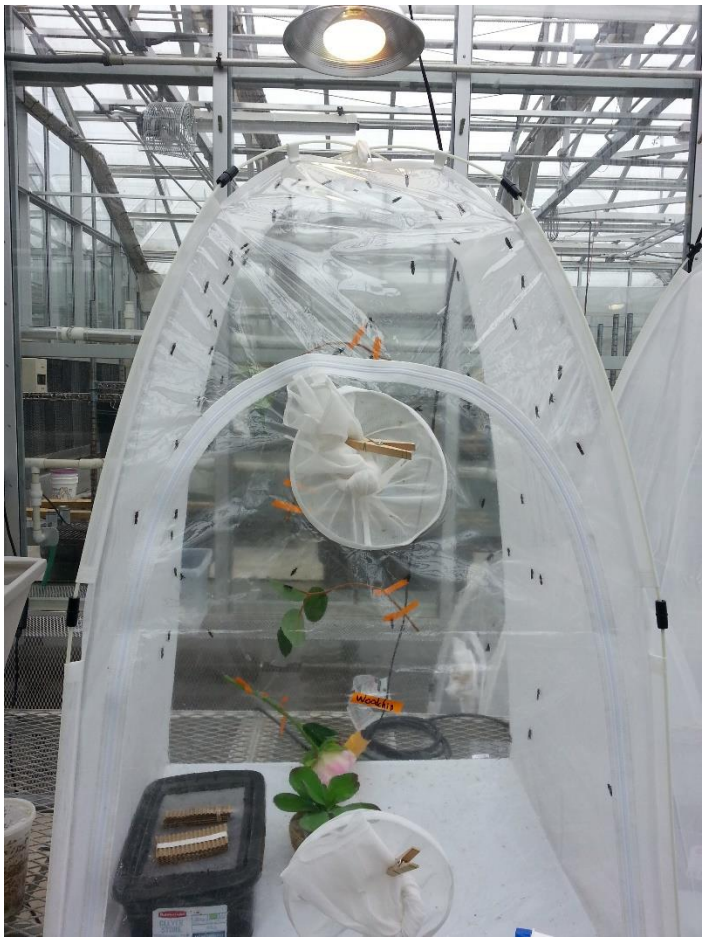

Figure 2. BugDorm™ tent

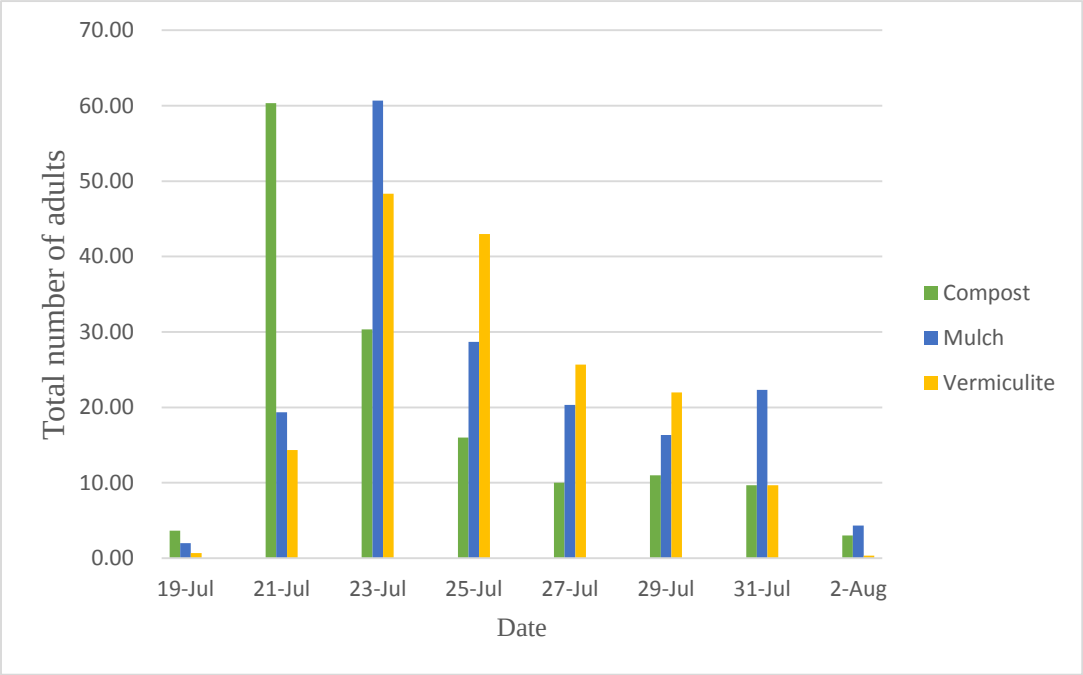

Figure 3. Adult emergence rates in mulch, compost and vermiculite media.

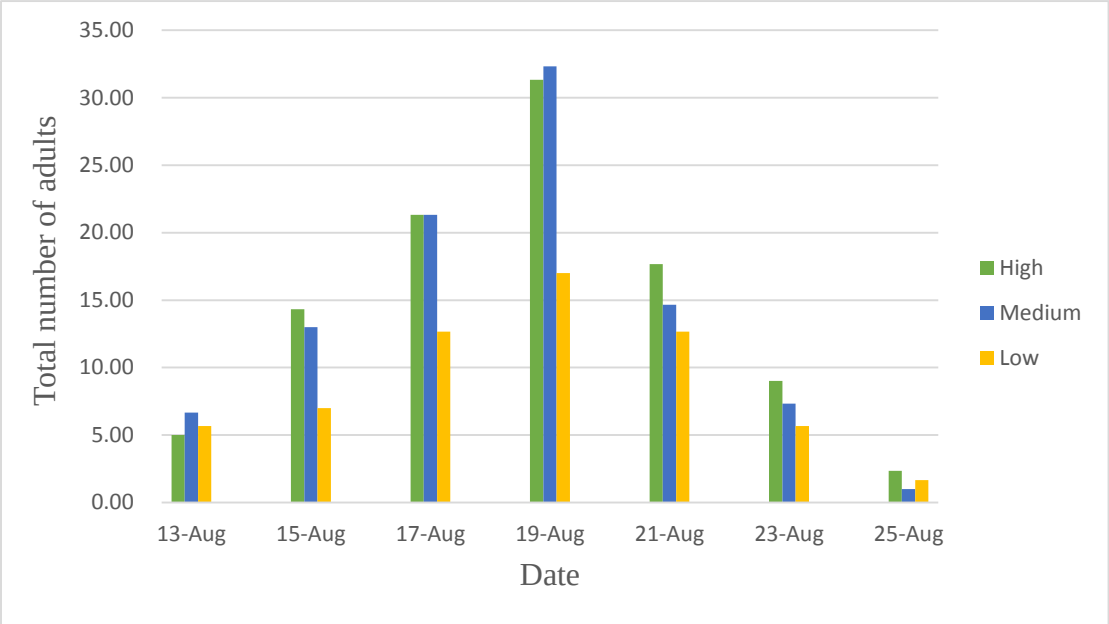

Figure 4. Adult emergence rates in mulch media at three moisture levels.

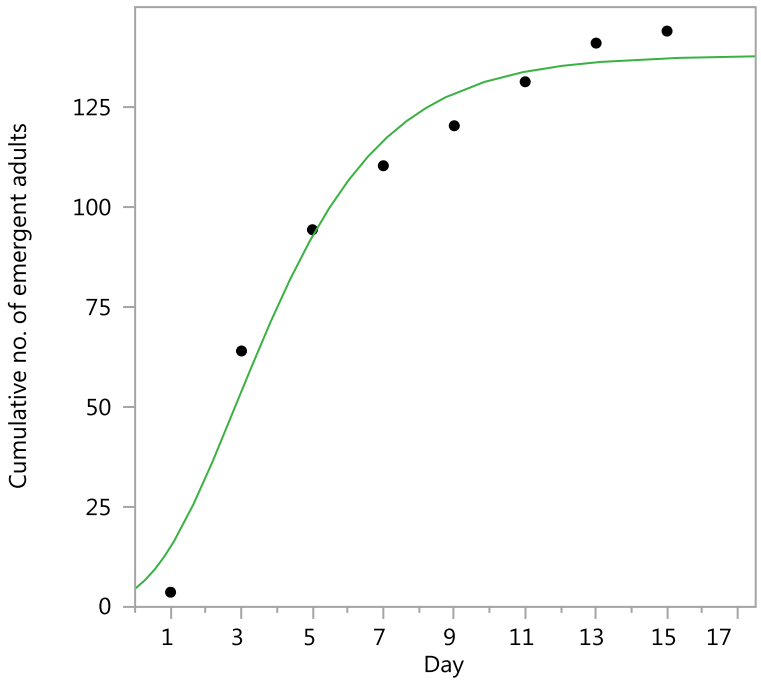

Figure 5. Compost media treatment adult emergence data fit with Gompertz 3P model curve.

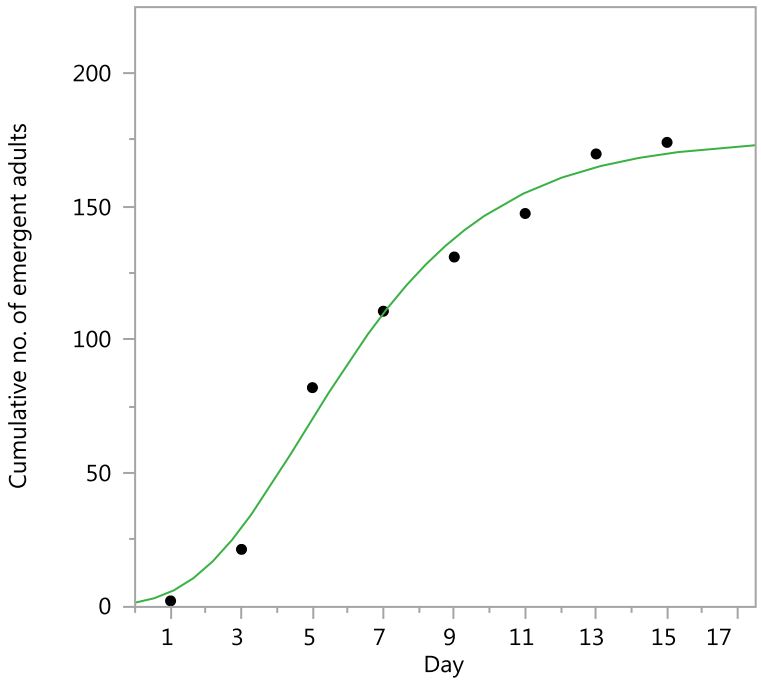

Figure 6. Mulch media treatment adult emergence data fit with Gompertz 3P model curve.

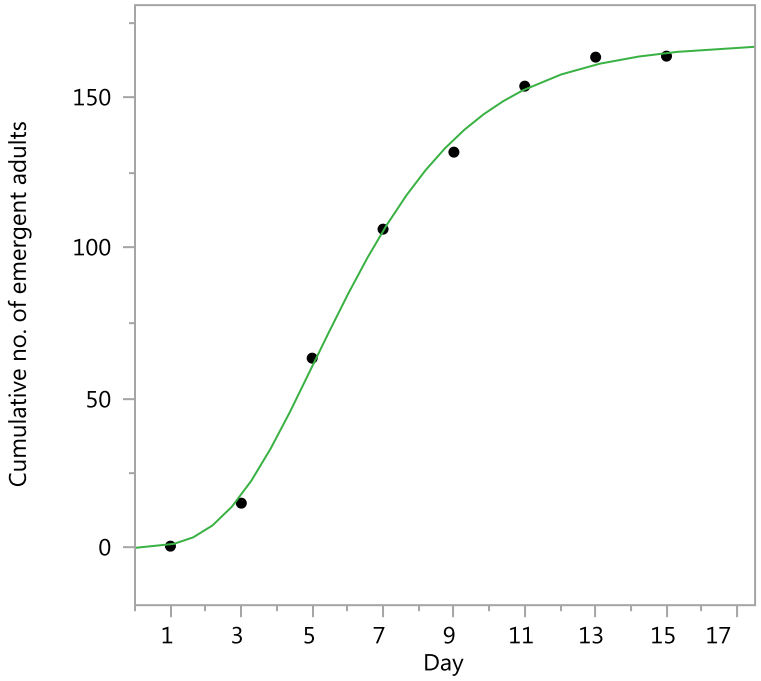

Figure 7. Vermiculite media treatment adult emergence data fit with Gompertz 3P model curve.

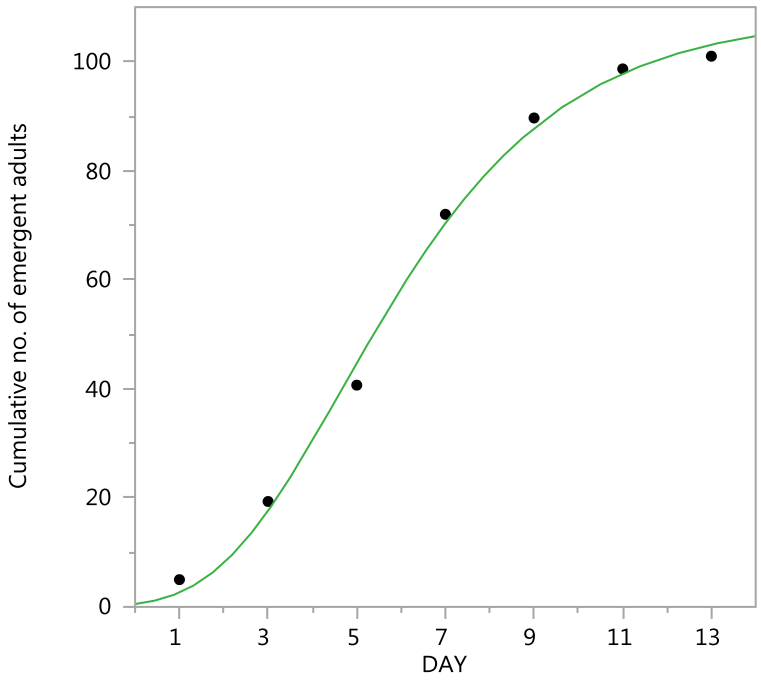

Figure 8. High moisture level adult emergence data fit with Gompertz 3P model curve.

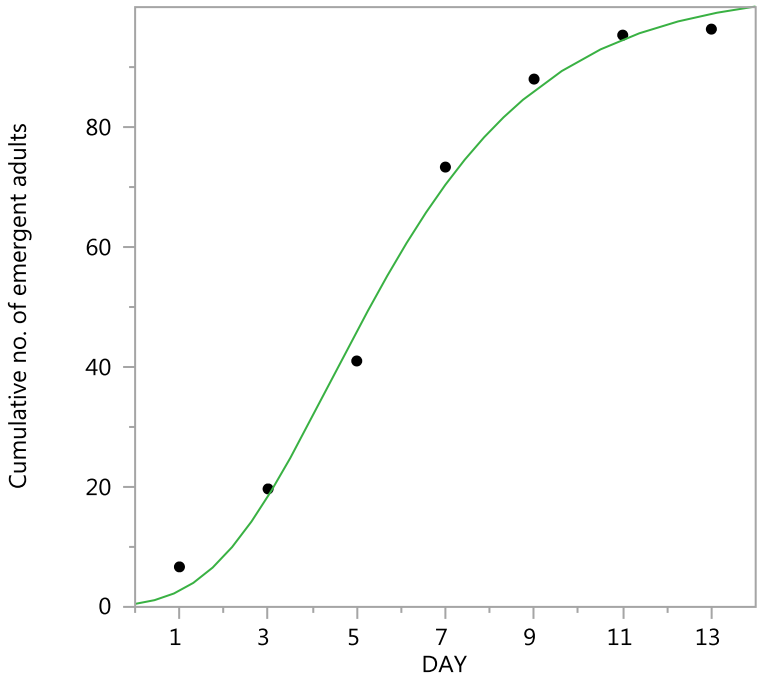

Figure 9. Medium moisture level adult emergence data fit with Gompertz 3P model curve.

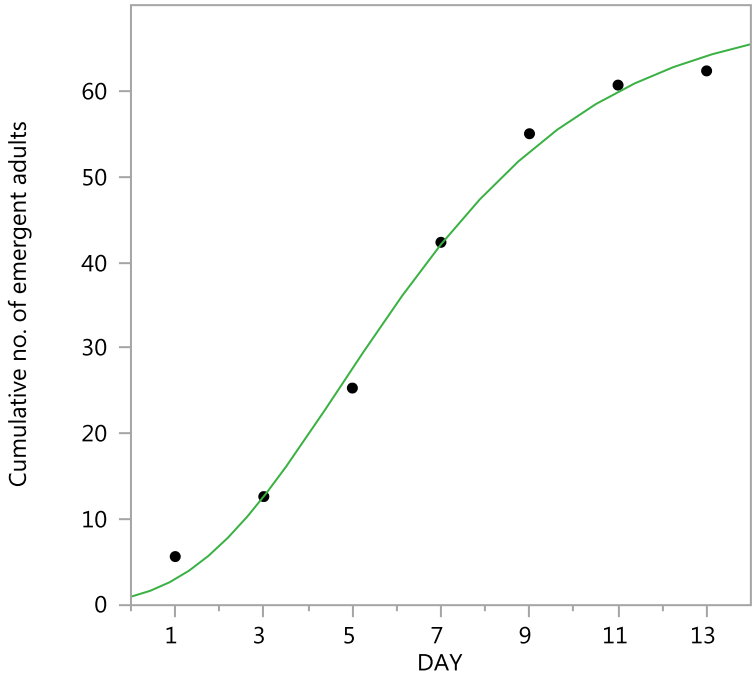

Figure10. Low moisture level adult emergence data fit with Gompertz 3P model curve.

Figure 11. Black soldier fly adult with malformed wings